main of the Notch receptor (NICD). These results prompted us to study the effects of Notch signaling after cytokine stimulation of articular chondrocytes.
Methods: Gene expression of several Notch markers in human articular chondrocytes were studied after TNF-α and IL-1β (10 ng/ml) stimulation for 24h using real-time PCR.
Results: Stimulation with either TNF-α or IL-1β resulted in almost the same effects on expression of Notch markers. Gene expression of Notch3, Jagged1, and HES5 were significantly decreased after stimulation with TNF-α or IL-1β. Notch1 was significantly repressed after TNF-α stimulation while its expression after IL-1β treatment only tended to decrease. Transcription of Delta4 was significantly increased after IL-1β stimulation and the expression slightly increased by TNF-α treatment.
Conclusions: This study shows that markers for the Notch signaling pathway in chondrocytes are affected by cytokine stimulation. Synoviocytes and chondrocytes respond differently to cytokine stimulation with regard to Notch signalling.

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INHIBITION OF MITOCHONDRIAL RESPIRATORY CHAIN ACTIVATES CICLOXYGENASE 2 PROTEIN EXPRESSION AND PROSTAGLANDIN 2 SYNTHESIS IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: In this study we investigated whether inhibition of mitochondrial respiratory chain (MRC) is able to modulate the inflammatory response in human osteoarthritic (OA) chondrocytes and the implication of stress inducing signals in this process.
Methods: Human OA chondrocytes were isolated from cartilage obtained from patients undergoing joint replacement. Rotenone, 3-nitropropionic acid (NPA), antimycin A, sodium azide and oligomycin were employed to inhibit complex I, II, III, IV or V of MRC, respectively. Flow cytometry was used to know the COX-1 and COX-2 protein expression as well as reactive oxygen species (ROS) liberation. PGE_2 production was evaluated by ELISA. NF-κB expression was quantified with a transcription factor assay. To demostrate the role of ROS, Ca^{2+} and NF-κB in COX-2 expression induced by inhibition of MRC, N-acetyl cysteine 2mM (a ROS inhibitor), Ruthenium Red 1 μM (an inhibitor of mitochondrial Ca^{2+} exhange), and BAY 117085 1μM (an inhibitor of NF-κB activation) were used.
Results: Firstly, we assesed the production of the inflammatory mediator PGE_2 in OA cells in the presence of rotenone (10-50 μg/ml), NPA (0.5-10 mM), antimycin A (20-60 μg/ml), sodium azide (2-25 mM) and oligomycin (5-100μg/ml). IL-1β (5 ng/ml) was employed as positive control. After 48 h of stimulation, only antimycin A and oligomycin increased PGE_2 (basal, 15.61±1.86; antimycin A, 96.90±12.72 and oligomycin, 64.03±20.29 pg/50.000cells). Secondly, we studied the percentage of cells that expressed COX-1 or COX-2 protein in the presence of all MRC inhibitors. Again, the inhibition of complex III and V showed an increase in COX-2 protein. After 5 h of stimulation, antimycin A produced a 32.24±4.68% of positive cells and oligomycin a 28.89±4.13% respect to basal (12.91±1.96). This expression was maintained after 18 h of stimulation. COX-1 didn't show any modulation. Thirdly, as mitochondrial complex activity downregulation could be by ROS or calcium exchange, we studied whether these mechanisms contributed to the increase of COX-2 expression and PGE_2 production. Notably, the percentages of cell population that produced H_2O_2 after treatment with antimycin A and oligomycin at 5 minutes were 63.11±6.03% and 58.35±6.51%, respectively vs basal (36.47±9.99%). Pretreatment for 2 h with N-acetyl cysteine (NAC) 2mM (a ROS inhibitor), or Ruthenium Red (RR) 1μM (an inhibitor of mitochondrial calcium exchange) diminished the effect of antimycin in the COX-2 expression up to 22.82% and 18.27%, respectively. Values for oligomycin decreased up to 47.29% and 47.68%. As NF-κB is one of the transcription factors implicated in COX-2 expression, we examined if it's required for the increase of COX-2 protein induced by MRC inhibitors. Our results showed that a NF-κB inhibitor, BAY 117085 (1 μM), reduced in a 17.26% the effect of antimycin A and a 36.97% the effect of oligomycin in the expression of COX-2 protein.
Conclusions: These results show that the inhibition of MRC complex III and V induces an inflammatory response in human OA chondrocytes, that could be mediated by ROS, mitochondrial calcium exchange and NF-κB activation.

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ELECTRON MICROSCOPIC STEREOLOGY IN THE ANALYSIS OF CARTILAGE COLLAGEN FIBRIL NETWORK OF TRANSGENIC MICE

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Purpose: To investigate by electron microscopic stereology the properties of cartilage collagen fibril network in newborn transgenic mice. The gene-mutated mice harbored transgenes targeted to affect the structure or assembly of the collagen fibrils in murine cartilage.
Methods: Cartilage of murine tibial growth plate or nasal septum were investigated by EM stereological technique using isotropic, uniform random (IUR) sampling with isector, and indirect estimation of stereological parameters, i.e. volume fraction (VV) and surface density (SV), using average fibril diameter and collagen length density (LV) (Langsjo et al. 1999 and 2002). Five types of genetically mutated mice were investigated and compared to their wild-type (wt) littermates. The following mouse lines were investigated: (i) mice with one active murine Col2a1 gene and two copies of human COL2A1 transgene with Arg519Cys mutation (M+/-H) (Li et al. 1995, Arita et al. 2002), (ii) mice with no active murine Col2a1 gene alleles and two copies of the human COL2A1 transgene with Arg519Cys mutation (M-/-H) (Li et al. 1995, Arita et al. 2002), (iii) mice harboring no active murine Col2a1 gene alleles and four copies of human COL2A1 transgene with Arg519Cys mutation (M-/-HH) (Li et al. 1995, Arita et al. 2002), (iv) mice harboring a copy of human COL2A1 transgene with deleted exons 16-27 and two wild-type Col2a1 alleles (M+/+COL2A1-Del) (Helminen et al. 1993), and (v) mice with inactive alleles of procollagen N-proteinase (PNP-KO) (Li et al. 2001). N-proteinase cleaves off the N-propeptides of procollagen monomers making the collagen molecule able to incorporate within the fibril.
Results: In all newborn (1 to 2 days old) mice carrying the COL2A1 transgene with Arg519Cys mutation (M+/-H, M-/-H, M-/-HH) the growth plate collagen fibrils were thinner (diam. 15 to 16 nm) than in wt mice (diam. 18 nm) and showed reduced volume fraction (%) of the fibril network ($p < 0.05$ - 0.001). On the other hand, in M+/+COL2A1-Del mice, the collagen fibril thickness remained unaltered but the volume fraction of collagen was reduced ($p < 0.05$). In PNP-KO mice, fibril thickness and the volume fraction of collagen did not differ from the wt mice.
Conclusions: The EM stereological technique proved to be efficient in revealing differences in the collagen fibril thickness and the collagen volume fraction in newborn mice harboring mutations which affected structure and assembly of collagen fibrils. This made possible early detection of the altered phenotype.